REMARKS

Claims 21-29 are pending in this application. Applicants respectfully submit that no new matter has been added. Reconsideration and allowance are respectfully requested.

Claims 21-23 have been amended to recite SEQ ID NO.:6. Claims 21, 24, 24 and 27 have been amended to add clarity. Support for the amendments can be found throughout the application as originally filed. Applicant respectfully submits that no new matter will be introduced via these amendments to the claims.

The specification has been amended to recite sequences identifiers at pages 2 and 20. The *Clostridium sordellii* lethal Toxin (LT) sequence referred to at page 2 of the application as "SEQ ID NO.: 6 is the published amino acid sequence of this toxin. Applicant respectfully submits that no new matter will be introduced via these amendments to the specification.

Applicant submits herewith a substitute Sequence Listing in both paper and computer readable format. The Sequence Listing is submitted in accordance with 37 C.F.R. §§ 1.821 et seq. As attested to in the attached Statement, the contents of the paper copy and computer readable copy of the sequence listing are the same. Entry of the substitute Sequence Listing into the record is respectfully requested.

The Examiner has objected to the Applicant's claim to priority, asserting that copies of the PCT and EPO parent documents of this application are not in the official Patent Office file. Applicant respectfully contends that it is impossible for a copy of the PCT parent document to be

absent from the file, as if this were true, the Examiner would have no application to examine on the merits.

The Examiner's attention is directed to the header set forth on each page of the instant application, which recites in the left margin " WO 97/27871" and in the right margin "PCT/EP97/00426". The instant application is a CPA of U.S. Application Serial No. 09/126,816, which was filed on July 31, 1998 as a Continuation Application of PCT/EP97/00426. Application PCT/EP97/00426, filed January 31, 1997, claims priority from EP Application 96101469.3, which was filed February 2, 1996. Because U.S. Application Serial No. 09/126,816 was filed as a Continuation Application, the identical specification, figures and claims filed in PCT/EP97/00426 were filed in U.S. Application Serial No. 09/126,816, as required by U.S. patent practice. Copies of the International Search Report and the published front page of corresponding application WO 97/27871 were included with the filing papers submitted to the USPTO on July 31, 1998. A copy of the date stamped receipt received from the USPTO showing the submission of these materials is included herewith.

In an effort to expedite prosecution on the merits, Applicant has obtained a certified copy of PCT/EP97/00426, although this expenditure of Applicant's resources seems to have been unnecessary. Acknowledgment of the receipt of this certified copy of the PCT application is respectfully requested, together with acknowledgement that the Examiner previously had a copy of the application as filed on July 31, 1998 by Applicant's agent.

With respect to the priority document for PCT/EP97/00426, Applicant was required under Rule 17.1 PCT to submit a certified copy of the priority

document to the International Bureau or receiving Office within 16 months of the claimed priority date. Where Applicant has complied with Rule 17.1 PCT, a designated Office may request that the International Bureau supply a copy of the priority document to that Office. The United States is a designated State, as evidenced by the cover page of WO 97/27871, meaning the USPTO is the designated Office for PCT/EP97/00426 upon entering National Phase in the United States. The Examiner's attention is directed to Rule 17.2(a) PCT, wherein it is expressly stated "[n]o such Office shall ask the applicant himself to furnish it with a copy [of the priority document]". Therefore, if the official Patent Office file lacks a copy of PCT/EP97/00426 priority document EP Application 96101469.3, the burden rests on the Examiner to acquire this priority document via appropriate channels.

Therefore, the Examiner's contention that the parent PCT and EP documents are missing from the instant application baffles Applicant. The Examiner is respectfully requested to review PCT Continuation practice rules and the contents of the instant application to be certain materials truly are missing from the Patent Office file. Should the Examiner continue to find deficiencies, the Examiner is encouraged to contact Applicant's agent by telephone to further clarify exactly what is needed from Applicant.

Claims 21-29 were rejected under 35 USC § 112, second paragraph, for allegedly being indefinite. The Examiner stated that the claims were indefinite in the recitation of the phrase "consisting essentially of approximately" the first 1020 N-terminal amino acids of the amino acid sequence of *Clostridium sordellii*. The Examiner also rejected these claims for referring to the amino acid sequence of SEQ IS NO.:1.

Claims 25-29 were rejected for reciting the phrase "A compound" according to one of claims 22 to 24.

Applicant traverses the Examiner assertions that claims are indefinite, and that a skilled artisan would understand how to practice the claims as originally written. Nevertheless, in an effort to expedite prosecution on the merits, Applicant has amended the claims for clarification purposes. Applicant respectfully submits that the claims are definite, and that this Section 112(2)-based rejection should be wtihdrawn.

The Examiner has rejected claims 21-24, 26 and 28 under 35 USC § 102(b) as being anticipated by Popoff or Roberts, as evidenced by Chaves-Olarte. Applicant traverses this rejection.

Applicants reiterate that Chaves-Olarte is not prior art to the instant application. As explained in Applicant's response filed July 5, 2000, the Chaves-Olarte reference was published April 1999. As discussed above, the instant application is a CPA of a Continuation Application of PCT/EP97/00426. The U.S. filing date for instant application Serial No. 09/126,816 is July 31, 1998, as acknowledged by the USPTO. *See* USPTO filing receipt for Serial No. 09/126,816, enclosed herewith. The Examiner's failure to explain why "reasons of record" preclude acknowledgment by the Patent Office that Chaves-Olarte is not prior art ot the subject application. The Examiner points to Paper 13, page 9 as support for the agency's position. However, Applicant notes that the Examiner relies upon an inaccurate statement for support, as paragraph (g) of page 9 asserts that Applicant's response filed July 5, 2000 states that Chaves-Olarte has a publication date after the *priority* date of the instant application. This statement is false, as

Applicant stated then, and continues to maintain that Chaves-Olarte has a publication date after the filing date of the instant application. Correction of the record by the Examiner is respectfully requested.

Popoff (1987) discloses a method of purifying active lethal toxin (LT) from *Clostridium sordellii*, and describes the serological relationship between *Clostridium sordellii* lethal toxin and *Clostridium difficile* lethal toxin. Popoff fails to teach a method of obtaining an active fragment of *Clostridium difficile* lethal toxin, and does not teach how to isolate an active fragment thereof. Rather, Popoff teaches that thiol groups that are essential for LT activity must be protected as disulfide bridges. *See* Popoff at Abstract. In addition, Popoff is silent with respect to the molecular targets or the enzymatic action of *Clostridium sordellii* lethal toxin.

Roberts discloses bacteria or bacterial components employed in preparing vaccines against several Clostridial species. The Examiner argues that the toxoids of Roberts' vaccines would inherently possessthe functional property of glycosylating activity. However, the Examiner offer s no susbtantive basis for asserting that the toxoids of Roberts consist essentially of the first 1020 amino acids of the *Clostridium sordellii* lethal toxin, and has been isolated to preserve glucosyltransferase activity. No suggestion is made within Roberts that the isolation of only a fragment of *Clostridium sordellii* lethal toxin would permit the toxin to retain activity and be suitable as a vaccine, much less having use in any other composition.

The Examiner asserts that Chaves-Olarte provides evidence of the presence of the catalytic domain, hydrophobic domain, and receptor binding domain in the *Clostridium sordellii* lethal toxin described by Popoff and

Roberts. However, as Applicants pointed out previously, the DNA sequence of *Clostridium sordellii* lethal toxin was not known at the time Popoff and Roberts were published. Each of Popoff and Roberts teach entire *Clostridium sordellii* lethal toxin proteins, but not the metes and bounds of isolated, active subdivisions thereof. Thus, the cited references are not enabling for obtaining the appropriately sized toxin fragment exhibiting only glucosyltransferase activity. Chaves-Olarte cannot provide evidence of this kind, since it is not prior art to the claims.

Accordingly, Applicants respectfully submit that the claims are not anticipated by Popoff and Roberts, and that the Section 102(b)-based rejection over these references should be withdrawn.

The Examiner has rejected claims 21-24, 26 and 28 under 35 USC § 102(a) or (b) as being anticipated by Green or von Eichel-Strieber, as evidenced by Chaves-Olarte. Applicant traverses this rejection.

Green was accepted by its publishers less than one year prior to the February 2, 1996 priority date of the instant application. The Examiner should be perfectly capable of properly assessing the relevance of this reference under the patent rules defining Section 102(a) and 102(b) rejections. According to the Examiner, Green discloses "the cytotoxic L-encoding gene of *Clostridium sordellii*", and that a highly conserved hydrophobic domain and a highly conserved carboxyl terminus are described. Green also teaches that these conserved domains must play some significant, but undefined, role in the activity of the protein. *See* Green at p. 60, column 1. Thus, no teaching is set forth in Green defining or teaching the isolation of a polypeptide fragment of *Clostridium sordellii* lethal toxin consisting essentially of the first 1020 N-

terminal amino acids of the toxin or a portion thereof that exhibits glucotransferase activity. Green is not enabling for the isolation of the claimed active fragments of the of *Clostridium sordellii* lethal toxin, and, accordingly, does not anticipate the claims.

The Examiner notes that the von Eichel-Streiber reference teaches "toxins of C. Difficile [sic] contain a amino terminal toxic domain, an intermediary translocation domain and a final C terminal region contributing to cellular binding". However, Applicant's claims are not directed to toxins of *C. difficile*, but a particular fragment of the *Clostridium sordellii* lethal toxin. The claimed fragment consists essentially of the first 1020 amino acids of the amino acid sequence of *Clostridium sordellii* lethal toxin, or a portion thereof, having glucosyltransferase activity. The von Eichel-Streiber reference is silent with respect to this and other aspects of the claims. Thus, von Eichel-Streiber cannot anticipate the claims.

Neither of Green or von Eichel-Streiber discloses the metes and bounds of isolated, active subdivisions *Clostridium sordellii* lethal toxin. Thus, the cited references are not enabling for obtaining the appropriately sized toxin fragment exhibiting only glucosyltransferase activity. Chaves-Olarte cannot provide evidence of this kind, since it is not prior art to the claims. Applicant respectfully submits that the claims are not anticipated by Green or von Eichel-Streiber, that that these Section 102-based rejections should be withdrawn.

In order to establish a *prima facie* case of anticipation, the Examiner must show that the cited references individually teach every element of the claims. *In re Bond*, 910 F.2d 831 (Fed. Cir. 1990). In order to state that the

cited references inherently possess the claimed elements, the Examiner must do more than make naked assertions. Inherency may not be established by probabilities or possibilities, and it is insufficient for the Examiner to assert, as is done here, that a certain thing may result from a given set of circumstances. *In re Oelrich and Divigard*, 666 F.2d 578 (C.C.P.A. 1981). Failing to show the enabled teachings of the claimed polypeptide fragments, the Examiner has not met this burden set forth by our courts for satisfying a rejection under Section 102. Therefore, none of the references cited by the Examiner qualify as anticipatory art for reasons discussed above.

The Examiner has rejected claims 21-29 under 35 USC § 103(a) as not being patentable over Popoff, or von Eichel-Streiber in combination with Blakey. The Examiner asserts that it would have been obvious for one of ordinary skill in the art to "chemically conjugate any of the prior art's Clostridium sordellii LT to antibodies or active fragments thereof directed against tumor associated antigens (TAA), as described by Blakely". *See* Paper 13 at page 12. Applicants traverse this rejection for at least the reasons of record and the following.

In making this obviousness-based rejections, the examiner relies on the teachings of Popoff and von Eichel-Streiber as primary references. However, as explained above, none of these references teaches the isolation of a particular fragment of the *Clostridium sordellii* lethal toxin exhibiting activity. No suggestion is made to cleave a portion of the *Clostridium sordellii* lethal toxin and utilize it with a pharmaceutically acceptable adjuvant or carrier. Moreover, no guidance is given as to which amino acids of the *Clostridium sordellii* lethal toxin would be capable of providing a fragment of

the toxin having glucosyltransferase activity. The secondary reference, Blakely, is silent with respect to fragments of *Clostridium sordellii* lethal toxin having glucosyltransferase activity, and adds nothing to supplement the deficiencies of the primary references.

By contrast, the claims are directed to a polypeptide fragment that consists essentially of the first 1020 amino acids of the amino acid sequence of *Clostridium sordellii* lethal toxin, or a portion thereof, having glucosyltransferase activity. The claimed polypeptide fragment may be included in a compound also having a target cell specific binding domain and a translocation domain. The claimed polypeptide fragment may also be included in a composition, together with a pharmaceutically acceptable adjuvant or carrier. Also claimed are methods of manufacturing the claimed composition.

The combination of references cited by the Examiner fail to enable the identification and isolation of a polypeptide fragment having glucosyltransferase activity. Furthermore, no suggestion is made by the references to utilize only a fragment of the *Clostridium sordellii* lethal toxin, or a portion thereof, having glucosyltransferase activity in compounds or compositions together with pharmaceutically acceptable adjuvants.

The Examiner has the initial burden of presenting a *prima facie* case of obviousness when making a Section 103 rejection. In Re Rijckaert, 28 U.S.P.Q.2d 1955, 1956 (Fed. Cir. 1993). A *prima facie* case of obviousness is established if the prior art suggested to one of ordinary skill in the art to modify the prior art in such a fashion as to produce the claimed invention, and

that such a modification would reasonably have been expected to succeed. In Re Dow Chemical, 5 U.S.P.Q. 1529, 1531 (Fed. Cir. 1988).

In the instant case, no motivation is provided within the cited references to modify the teachings thereof in order to obtain the claimed subject matter. Moreover, nothing in the references suggests that a skilled artisan would expect such modifications to be successfully implemented based on the teachings of Popoff, von Eichel-Streiber with or without Blakely. Accordingly, the Examine has failed to meet the requirements for establishing a prima facie case of obviousness as set forth by Dow Chemical. Applicant respectfully submits that the claims are patentable over the cited art, and that the Section 103-based rejection should be withdrawn.

In view of the foregoing amendments and remarks, it is respectfully submitted that the application is in condition for allowance. Notification to that effect is respectfully requested. Should any questions related to patentability remain, the Examiner is invited to telephone the undersigned to discuss the same.

Respectfully submitted,

PILLSBURY WINTHROP LLP

By Blair Elizabeth Taylor

Blair Elizabeth Taylor, Ph.D.
Registration No. 44,370
Tel. No.: (703) 905-2198
Fax No.: (703) 905-2500

1600 Tysons Boulevard
McLean, Virginia 22102
(703) 905-2000

Enclosure: Copies of date stamped filing post card receipt, USPTO filing receipt, Sequence Listing (CRF and paper copies), and certified copy of PCT.

APPENDIX
MARK UP VERSION SHOWING CHANGES MADE

IN THE SPECIFICATION:

The specification has been amended as indicated below.

At page 2, the paragraph beginning at line 7has been amended as follows:

Several different species of the genus Clostridium produce large molecular weight (250-300 kDa) cytotoxins which cause effects on the actin cytoskeleton, including disruption of actin stress fibers and rounding up of cell bodies. This sub-group of clostridial cytotoxins includes toxins A and B from *Clostridium difficile*, lethal toxin (LT) (SEQ ID NO.: 6) and hemorragic toxin (HT) from *Clostridium sordellii*, and *Clostridium novyi* α-toxin (Bette, P., et al., Toxicon 29 (1991) 877-887). Enterotoxin A and cytotoxin B have been characterized by Sullivan, N.M. et al., Infect. Immun. 35 (1982) 1032-1040, von Eichel-Streiber, C., et al., Microbiol. Pathogenesis 2 (1987) 307-318. Toxin A and toxin B are glucosyltransferases which modify threonine 37 of the GTPase Rho. By attracting of glucose at this position of Rho, this GTPase is blocked in its function. Recently, toxin B and toxin A from *C. difficile*, the causative agent of antibiotic-associated diarrhea (Lyerly, D.M., et al., Clin. Microbiol. Rev. 1 (1988) 1-18), were shown to covalently modify the mammalian protein Rho by UDP-Glc dependent glucosylation of threonine 37 (Just, I. et al., Nature 375 (1995) 500-503; Just, I., et al., J. Biol. Chem. 270 (1995) 13932-12936). Rho is a small ras related GTP-binding protein involved in the control of actin polymerization (Hall, A., Ann. Rev. Cell Biol. 10

(1994) 31-34). Glucosylation of threonine 37 of Rho by *C. difficile* toxins A or B apparently inactivates this protein and results in a loss of actin stress-fiber assembly.

At page 20, the paragraph beginning at line 4 has been amended as follows:

To identify the acceptor amino acid glucosylated by LT, H-Ras protein as modified by LT in the presence of UDP-[^{14}C] Glc, electrophoresed on SDS-PAGE, digested with trypsin and the resulting peptides were separated, as described in sections 1 and 2. As shown in Fig. 4A, 47 fractions were obtained. The radioactivity was exclusively associated with fractions 39 and 40 (Fig. 4A). As shown in Fig. 4B and 4C, repurification of fraction 39 or 40 gave rise to a major peptide (D for 39 and E for 40) containing the radioactivity and several other small peptides. Peptides D and E were microsequenced and gave exactly the same amino-acid sequence. Each cycle of Edman degradation was collected and counted for radioactivity. The following unambiguous sequence was found for these peptides SALTIQLIQNHFVDEYDPTIEDSYR (SEQ ID NO.: 5). Cycle 19 corresponding to a threonine gave a very small signal. The small amount of threonine detected in position 19 may be the consequence of the LT catalyzed glucosylation of most of Ras molecules present in the reaction. Decrease or absence of threonine 37 Rho A in automated amino-acid sequencing, after glucosylation by toxin A or B, has been already reported (Just, I. et al., Nature 375 (1995) 500-503; Just, I., et al., J. Biol. Chem.. 270 (1995) 13932-13936). The amino-acid sequence found for both peptides D and E corresponds

exactly to a sequence found in the H-Ras protein between amino-acids 17 to 41 (Barbacid, M., Ann. Rev. Biochem. 56 (1987) 779-827). Radioactivity was associated first with cycle 19 and decreased thereafter (Fig. 4E). The rise in radioactivity at cycle 19 establishes threonine 35 (of the H-ras molecule) as the unique amino-acid glucosylated by LT.

IN THE CLAIMS:

The claims have been amended as indicated.

21. (Amended) An isolated [A] polypeptide fragment of *Clostridium sordellii* lethal Toxin (LT) with glucosyltransferase activity, consisting essentially of approximately the first 1020 N-terminal amino acids of the amino acid sequence of *Clostridium sordellii* lethal Toxin (LT) according to [SEQ ID NO:1] SEQ ID NO:6, or a portion thereof having glucosyltransferase activity.

22. (Amended) A compound comprising a polypeptide fragment of *Clostridium sordellii* lethal Toxin (LT) consisting essentially of approximately the first 1020 amino acids of the amino acid sequence of *Clostridium sordellii* lethal Toxin (LT) according to [SEQ ID NO:1] SEQ ID NO:6, or a portion thereof, the compound having (i) a glucosyltransferase activity domain, and (ii) a target cell specific binding domain which permits the compound to bind to a target cell.

23. (Amended) A compound comprising a polypeptide fragment of *Clostridium sordellii* lethal Toxin (LT) consisting essentially of approximately the first 1020 amino acids of the amino acid sequence of *Clostridium sordellii*

lethal Toxin (LT) as defined by [SEQ ID NO:1] SEQ ID NO:6, or a portion thereof, the compound having (i) a glucosyltransferase activity domain, (ii) a target cell specific binding domain, which domain causes the compound to bind to a target cell, and (iii) a translocation domain for translocating a catalytic domain of *Clostridium sordellii* lethal Toxin (LT) from the exterior of a cell into the interior of said cell.

24. (Amended) The [A] compound according to claim 23, wherein the translocation domain consists essentially of approximately the N–terminal amino acids 1021-1700 of the amino acid sequence of *Clostridium sordellii* lethal Toxin (LT).

25. (Amended) The [A] compound according to one of claims 22 to 24, wherein the target cell specific binding domain is an antibody or an antigen binding fragment thereof.

27. (Amended) The [A] composition comprising a compound according to claim 25 and a pharmaceutically acceptable adjuvant or carrier.